Reprinted from *Crop Science*
Vol. 34, No. 4

Mycorrhizae Alter Protein and Lipid Contents and Yield of Pea Seeds

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ABSTRACT

Root colonization by arbuscular-mycorrhizal (AM) fungi may affect seed protein and lipid composition by altering P nutrition or by eliciting metabolic responses by the host plant. These fungi may therefore play a role in plant breeding programs. This study was conducted to determine the effects of an AM fungus and different levels of P availability on seed protein and lipid composition and yield. Pea (*Pisum sativum* L.) plants were grown in a greenhouse under different P regimes (0, 1, 2, or 4 g hydroxyapatite kg^{-1} soil) with or without the AM fungus *Glomus mosseae* (Nicol & Gerd.) Gerd. and Trappe. At the lowest level of P availability, protein concentration was significantly lower and lipid concentration and seed dry mass were higher in AM than in non-AM plants. Protein/lipid concentration ratios were invariant in non-AM plants at all soil P levels. Those of the AM plants varied, were highest at an intermediate P level, and coincided with the highest intensity of root colonization and the greatest reduction of seed yield relative to the non-AM plants at the same level of P availability. Lipid and protein contents were highly correlated (second order) with P content in all plants. In non-AM plants, however, lipid and protein contents were very low at the lowest soil P level, but statistically not different at the other soil P levels. The data show different patterns of seed P accumulation and different relationships between seed P content and protein and lipid composition in AM and non-AM plants. This suggests that both the presence and the intensity of AM-fungal colonization altered the response of seed lipid metabolism to increasing P availability, which affected the protein and lipid ratios.

ARBUSCULAR-MYCORRHIZAL FUNGI have fundamental effects on host-plant biochemistry and physiology (Koide and Schreiner, 1992; Smith and Gianinazzi-Pearson, 1988) that are not part of the ecophysiology and plant-soil biology (Bethlenfalvay and Linderman, 1992) of the AM symbiosis. The production of symbiosis-specific proteins (Gianinazzi-Pearson and Gianinazzi, 1989), amino acid fractions (Krishna and Bagyaraj, 1983), lipids (Pacovsky, 1988), and secondary metabolites (Morandi and Gianinazzi-Pearson, 1986) by plants as a result of root colonization by AM fungi point to the manifold nature of the host response. Such changes found so far in other plant tissues suggest that seeds may also be modified in the mycotrophic plant (Lu and Koide, 1991), not only in terms of biomass produced, but also in the relative abundance of some of their main storage products: proteins and oils.

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Published in Crop Sci. 34:998–1003 (1994).

Abbreviations: AM, arbuscular-mycorrhizal; HAP, hydroxyapatite; Pr/L, protein/lipid.

Seed oil and protein composition are not simply inherited traits, and a large component of variation found in their expression may be ascribed to environmentalaction (Burton, 1987; Wilson, 1987). Since AM fungi are a dominant aspect of a plant's environment, evidence of their effects on the expression of genetic differences in host plants (Toth et al., 1984) also suggests a role in seed physiology. However, information on AM effects on seeds is scarce. This lack of data is due to the fact that most experiments with AM fungi are done in pot cultures utilizing initially-sterile soils needed for non-AM controls. Pot experiments are usually terminated before the plant matures because of rooting-volume constraints (Koide, 1991a) that limit AM effectiveness. Perhaps the most pronounced aspect of AM effectiveness is enhanced P nutrition (Koide, 1991b), and plant P status is known to both increase and decrease seed protein/lipid (Pr/L) balances (Jones and Lutz, 1971; Sawan et al., 1988). Seed Pr/L ratios may therefore be affected by AM fungi by at least two mechanisms: through enhanced P uptake, or through the elicitation of changes in seed Pr/L metabolism independent of P status. Based on these assumptions, AM technology may be applied to modify seed Pr/L ratios by the selection of fungal isolates that are effective in P uptake or in modifying seed Pr/L metabolism. It is therefore of interest to determine if the two putative mechanisms, in fact, function independently.

These mechanisms were evaluated by measuring the effects of the AM fungus *G. mosseae* on pea seed development and protein and lipid contents under different soil P regimes and by comparing the results with those found in non-AM plants.

MATERIALS AND METHODS

Experimental Design

The experiment consisted of eight treatments in a two × four factorial design with five replications. The treatment units (40) were potted pea plants. They were arranged in a completely random manner and rotated weekly. The factors were the symbiotic status of the plants (AM or non-AM) and four levels of P fertilization. The significance of differences between treatment parameters was evaluated by analysis of variance (main effects and interactions). Effects found significant were further tested by Duncan's multiple range test for the P regimes ($P < 0.05$) and Student's *t*-test. Actual probability values instead of the arbitrary 5% level are presented for AM vs. non-AM comparisons to give a more precise reading of the probability and to permit an individual interpretation of its significance (Nelson, 1989).

Biological Materials and Soil

Pea (cv. Little Marvel)[1] seeds were germinated, selected for uniformity, and planted in 1.5-L plastic pots. A sandy-loam soil obtained from the bank of the Willamette River near Corvallis, OR, was utilized. The steam-sterilized (100 °C) soil (pH 6.5, 1.7 kg per pot) had a texture of 71% sand, 20% silt, and 9% clay, and contained (g kg^{-1}): NH_4-N, 1.9; NO_3-N, 24.1; P ($NaHCO_3$-extractable) 0.010; P (total), 0.5; K, 176; Ca, 8.8; Mg, 3.5; S, 0.8; and micronutrients (mg kg^{-1}): B, 0.1; Cu 2.4; Fe 70.0; Mn 5.1; and Zn, 0.8. Phosphorus was added by mixing 0, 1, 2, or 4 g (kg^{-1} of soil) of finely-ground hydroxyapatite (HAP, $Ca_{10}[PO_4]_6[OH]_2$) into the soils of each pot before planting. A soil inoculum (50 g) of the AM fungus *G. mosseae*, WRRC isolate no. 1 (Franson and Bethlenfalvay, 1989) from pot cultures of *Sorghum* was mixed with the soil of the AM plants. It contained ≈20 sporocarps per gram of soil and a number of heavily colonized root fragments. A filtrate (100 mL) of the inoculum free of AM propagules was applied to both AM and non-AM soils.

Growth Conditions

Plants were grown in a greenhouse at Corvallis (April-June 1992). Automatic controls kept temperatures within a range of 20 to 30 °C in the greenhouse. Sunlight was supplemented by 1000 W phosphor-coated metal halide lamps (General Electric) in parabolic reflectors that extended daylight to 14 h and provided light of 500 µmol m^{-2} sec^{-1} at soil level. Plants were watered with tap water twice a week to field capacity. After the first week, a complete (but P-free) nutrient solution was also given once a week. This solution was adjusted to provide a relationship between macro-nutrients similar to that of Hoagland's solution (N:Ca:K:Mg:S, 16:4:6:1:1). Nutrient concentrations (m*M*) and total amounts of nutrients (g·kg $soil^{-1}$) added during the experiment were: $Ca(NO_3)_2$: 4, 2; KNO_3: 6, 1.3; NH_4NO_3: 1, 0.2; $MgSO_4$: 1, 0.5, respectively. Micronutrients were given as half-strength Hoagland's solution. Nitrogen, at the high concentration (16 m*M*) of the solution, completely inhibited nodulation.

Harvest and Assays

Roots obtained in soil cores were cleared and stained with trypan blue when the pods were beginning to dry (10 wk). Percent colonization by the AM fungus was then estimated by the grid-line intersect method (Giovanetti and Mosse, 1980). The plants were kept in the greenhouse for two more weeks until all pods were dry and seeds were mature. Seed protein (Automated Buchi-Kjeldahl Nitrogen Analyzing System, Brinkman Instruments, Westbury, NY), crude fat (ether extract, direct system), and P (Inorganic Phosphorus Test Kit, Sigma Diagnostics, St. Louis, MO; catalog no. 670-C) concentrations were determined from the pooled seeds of each plant by the Bioanalytical Services Laboratory, Department of Agricultural Chemistry, Oregon State University (AOAC, 1980).

RESULTS

Seed Yield and Phosphorus

Increased P availability through HAP addition to the soil resulted in increased seed yield in both AM and non-AM plants. The greatest difference ($P < 0.001$)

[1] Mention of a cultivar or brand name does not imply endorsement by USDA-ARS.

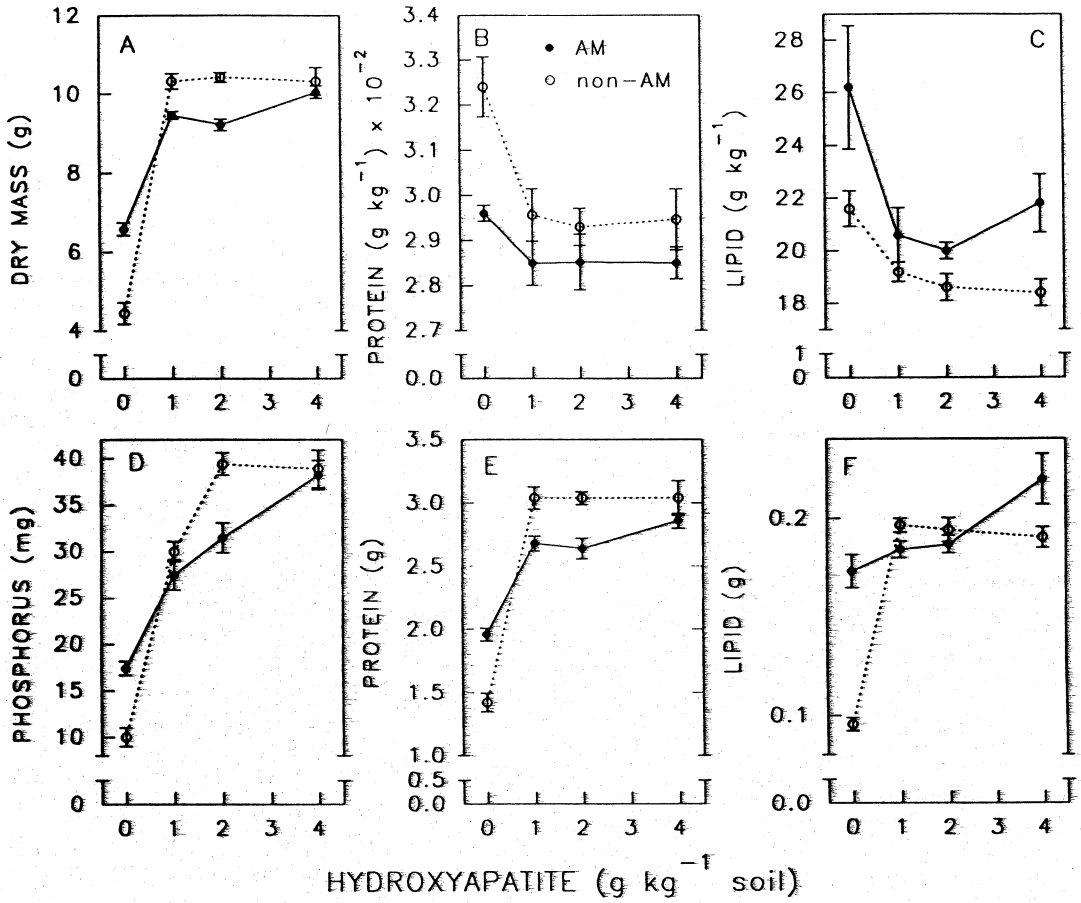

Fig. 1. Seed dry mass, seed protein and lipid concentrations, and seed P, protein and lipid contents of arbuscular-mycorrhizal (AM) and non-AM pea plants grown under different P regimes (0, 1, 2, or 4 g hydroxyapatite added per kg of soil). Data points represent the means (with standard error bars) of five replications.

between AM and non-AM plants was observed at the lowest (no HAP added) P level (Fig. 1A). Seed P contents increased gradually with each additional increment of P for AM plants, and more rapidly (except for the high-P treatment) for non-AM plants (Fig. 1D). The effects of AM colonization on seed development may best be visualized by considering the AM plant data relative to those of the non-AM control plants (Fig. 2). The data showed seed yield enhancement at the lowest P level, inhibition at the intermediate levels, and no response to the AM fungus at the highest level. Relative seed P concentrations showed a pattern of change with soil P similar to that of seed dry mass (Fig. 2).

Seed Protein and Lipid Composition

Seed protein and lipid concentrations diverged most at the 0-HAP level in AM and non-AM plants, with protein concentration lower ($P = 0.002$) and lipid concentration higher ($P = 0.059$) in the seeds of AM plants than in those of non-AM plants (Fig. 1B and 1C). At the higher HAP levels, protein concentrations in all seeds were lower, and there were no differences between AM and non-AM seeds ($P > 0.2$). Seed lipid concentrations were also lower for all plants at higher HAP levels, and

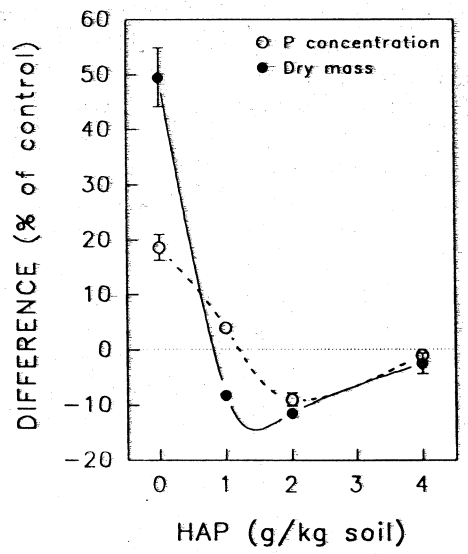

Fig. 2. Seed dry mass and seed P concentration of arbuscular-mycorrhizal (AM) pea plants grown under different P regimes [0, 1, 2, or 4 g hydroxyapatite (HAP) added per kg soil]. Values are expressed as the difference of percent of control [(AM − non-AM)/non-AM] × 100. Data points represent the means (with standard error bars) of five replications.

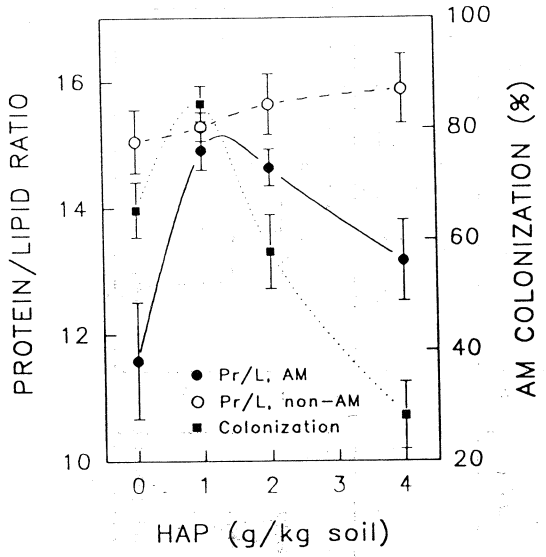

Fig. 3. Seed protein and lipid concentration ratios and root colonization of arbuscular-mycorrhizal (AM) and non-AM pea plants grown under different P regimes [0, 1, 2, or 4 g hydroxyapatite (HAP) added per kg soil]. Data points represent the means (with standard error bars) of five replications.

differed for the AM vs. non-AM plants in the seeds of 2-HAP ($P = 0.085$) and 4-HAP ($P = 0.006$) treatments.

The seed Pr/L ratios of non-AM plants were statistically invariant at all P levels but tended to increase with soil P (Fig. 3). Those of the AM plants, however, varied significantly, and reached their highest point near the range of soil P content where root colonization (Fig. 3) and the inhibition of seed production (Fig. 2) were maximal. Thus, the Pr/L ratio can serve as an indicator of AM effects, as illustrated by (i) the opposite trends of the Pr/L ratio (Fig. 3) and relative (AM vs. non-AM) seed yield (Fig. 1) and (ii) the similarity between the changes in root colonization and in the Pr/L ratio with enhanced P status (Fig. 3).

Seed P, protein, and lipid contents responded differently to changing soil P in AM and non-AM plants (Fig. 1D, 1E, and 1F) as evidenced by significant interactions ($P < 0.001$) between AM status and P fertilization. Seed P content was highly correlated (quadratic for best fit) with seed lipid and protein content in both AM and nonAM plants (Fig. 4). In AM plants, seed protein ($r = 0.9863$, $P = 0.0019$) and lipid contents ($r = 0.9996$, $P < 0.0001$) changed gradually with seed P content and continued to increase within the range of soil P fertilization. In non-AM plants, the change in seed protein ($r = 0.9999$, $P < 0.0001$) and lipid contents ($r = 0.9995$, $P < 0.0001$) with seed P content was abrupt. Seed protein and lipid contents were very low at the lowest level of seed P content (0 HAP treatment), doubled with the first HAP addition, but did not increase further regardless of the amount of HAP added.

DISCUSSION

There are two modes of action for adaptation of plants to the environment: genetic differentiation and phenotypic plasticity of the individual plant in response to environmental stresses (Kuiper, 1984). Root colonization by AM fungi may affect both modes simultaneously. Substantial diversion in mycotrophic associations of host photosynthate to fungal lipids is accompanied by characteristic alterations in the lipid metabolism of host tissues, and by a redistribution of carbon among lipid and nonlipid fractions of tissues throughout the plant (Lösel, 1980). The changes that occur in seed lipid content and composition when AM fungi are substituted for P fertilizer as the source of P nutrition suggest that genetically-based mechanisms play a role in the AM host response in addition to those due to changes in P nutrition or to relief from P stress (Pacovsky and Fuller, 1987). Although mineral nutrition does not seem to affect seed oil composition greatly in comparison with vegetative tissues (Hitchcock and Nichols, 1971), environmental conditions do affect seed protein and lipid composition (Dornbos and Mullen, 1992; Kuiper, 1984). The effects of improved P nutrition on seed lipid content are not

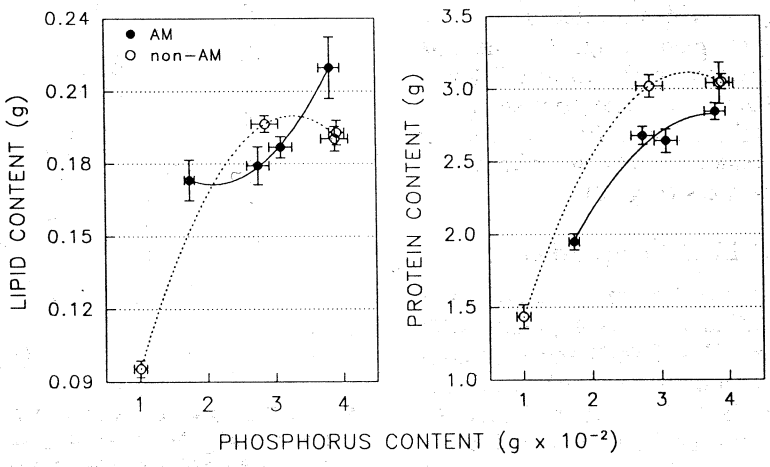

Fig. 4. Relationship of lipid or protein content with P content in seeds of arbuscular-mycorrhizal (AM) and non-AM pea plants. Data points from left to right reflect increasing soil P availability and represent the means (with standard error bars) of five replications. Lines represent highly significant second-order correlations (r values > 0.98, $P < 0.002$).

consistent, showing a decrease in soybean (Jones and Lutz, 1971) and an increase in cotton (Sawan et al., 1988).

Our data indicate that AM colonization affects the patterns of protein and lipid composition in pea seeds with changing soil P availability, and that this pattern is modified by the intensity of colonization. The continuous response of seed P accumulation to P availability and its correlation with protein and lipid contents in AM plants contrasted with the abrupt saturation pattern at low P availability in the non-AM plants. Likewise, the lack of response of seed Pr/L concentration ratios to P availability in the non-AM plants, and a strong response in the AM plants (Fig. 3), indicate that the mechanisms by which P nutrition affects the production of proteins and lipids in seeds is influenced by the symbiotic condition of the plant, and is not a function of P nutrition or P availability alone.

The depression of seed production and P concentration coincided with intensive colonization at intermediate soil P levels (Fig. 2) and was reminiscent of an inhibition of plant growth by AM fungi observed previously in whole plants (Bethlenfalvay et al., 1983). Such negative, or parasitic, host responses have been interpreted as competition for carbon (Fitter, 1991) by the symbionts. It remains to be seen, to what extent changes in carbon metabolism elicited by the AM condition are responsible for the host responses in seed protein and lipid status as observed here (Fig. 1). The carbon cost of supporting the endophyte, however, does not explain the differences in AM and non-AM seed composition at different levels of soil P availability or seed P content. With the satisfaction of sink demand by the endophyte as a dominant factor, lipid production (seed lipid content) should have been consistently lower in all AM plants, which was not the case. Different relationships between seed lipid and protein contents (Fig. 1) in AM and non-AM plants suggest an AM effect on seed composition distinct from the one mediated by P nutrition.

In the past, yield rather than seed oil or protein composition was the decisive factor in the selection for planting of cultivars of legume crops such as soybean (Fehr, 1987). However, shifting markets and a demand for specific products (Hurlburgh et al., 1990) may call for further improvement and refinement of the breeding process. Since the extent of possible improvement in the percentages of protein and oil in the seed is determined by the amount of genetic and environmental variation and their interaction (McKendry and McVetty, 1985), and since AM-fungal isolates differ markedly in their symbiotic effectiveness with their host (see Johnson and Pfleger, 1992), breeding strategies should consider and account for the AM fungus and its impact both on the genetic expression and the environment of its host plant. Utilization of AM fungi in plant breeding would call for the selection of isolates that elicit desired plant responses.

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